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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: BURNS et al.

Serial No.: 09/787,323 Group Art Unit: 1655

Filed: March 16, 2001 Examiner: Johannsen, Diana B.

For: *Multiple Sequencing Method*

Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO OFFICE ACTION

Sir:

This communication is responsive to the Office Action mailed 5 December 2001 in the above referenced patent application. Claims 1-13 are currently pending.

Please Amend the Specification as indicated below and shown in the subsequent pages.

In the Description:

On page 5 line 23 Please add the phrase "SEQ ID NO. 1" before first sequence on line 23.

Also on page 5, please add the phrase "SEQ. ID NO. 2" before the second sequence starting on line 23 and continuing onto line 24.

In the Claims:

Please amend claims 1-11, as indicated in the following pages and shown in the Appendix.

Please cancel claims 12 and 13.

Please add new claim 14.

Replacement Page

Analysis may be performed by any means desired. For example, analysis of gel electrophoresis, analysis on a capillary apparatus, or analysis by mass spectrophotometry can be performed.

Also provided is a kit for performing multiplex analysis of sequencing reactions comprising: an enzyme that cuts at least I base downstream of a selected-enzyme recognition site; and a set of oligonucleotide linkers comprising a recognition site for the selected enzyme. For example, the enzyme can be Bpm I, Bsg I, Eco57,or MmeI or a combination thereof The kit can further comprise, for example, a vector io for constructing a library wherein, for example, the vector has an appropriate cloning site for use in the method. The kit can further comprise a component to facilitate the multiplexing of the sequence reaction products, selected according to the analysis method to be used.

Examples

cDNA library construction. Polyadenylated RNA was isolated from 5 x 107 THP1 cells using FastTrack 2.0 (Invitrogen, San Diego, CA). A random oligomer primed cDNA library was constructed from 5 ~Lg of the polyA-selected mRNA using the Copy Kit (Invitrogen). E. coli DNA ligase was removed from the second- strand synthesis reaction to enhance synthesis of products approximately 900 base pairs in length. Next, BpniI linkers (SEQ. ID. NO 1 5'-AATTCGGCTCGAGCTGGAG-3' and SEQ. ID No. 2 5'- CTCCAGCTCGAGCCG-3') were added to the ends of the blunt-ended cDNA fragments using T4 DNA ligase. Following the addition of the linkers, the fragments were phosphorylated (T4 DNA kinase) and size selected using a Chromaspin 400 column (Clontech, Palo Alta, CA). The cloning vector pYesTrp2 (Invitrogen) was digested using the restriction endonuclease EcoRl at 37 'C. The linearized vector was dephosphorylated with shrimp alkaline phosphatase (SAP, Boerhinger Mannheim) prior to gel purification. CDNA inserts and treated, linearized vector DNAs were ligated into the cloning vector and the litigation product was transformed into